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Severe Impairment in Early Host Defense Against *Listeria monocytogenes* in Mice Deficient in Acid Sphingomyelinase

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The phagolysosomal compartment is crucial for the defense against infection with intracellular pathogens. Within this compartment, the TNF- and IFN-γ-responsive acid sphingomyelinase (ASMase) generates the signaling molecule ceramide, resulting in the activation of proteases like cathepsin D. To investigate the possible role of ASMase as a mediator of the antibacterial effects of TNF and IFN-γ, ASMase$^{-/-}$ mice were infected with *Listeria monocytogenes*. ASMase$^{-/-}$ mice showed a dramatically increased susceptibility to *L. monocytogenes* (LD$_{50}$ = 100 CFU) when compared with syngeneic wild-type mice (LD$_{50}$ = 10,000 CFU). In *L. monocytogenes*-challenged ASMase$^{-/-}$ mice, IFN-γ serum levels as well as IL-1β and IL-6 secretion by macrophages were similar to those observed in wild-type C57BL/6 mice. Although macrophages and granulocytes from ASMase$^{-/-}$ mice showed intact production of reactive nitrogen intermediates and oxidative burst, ASMase$^{-/-}$ macrophages were not fully incapable of restricting the growth of *L. monocytogenes* in vitro. The results of this study suggest that ASMase is crucially required for the intracellular control of *L. monocytogenes* in macrophages and granulocytes by nonoxidative mechanisms. *The Journal of Immunology*, 2003, 170: 2621–2628.

The infection of mice with *Listeria monocytogenes* is one of the best-studied immunologic paradigms that contributed to unraveling many important mechanisms of innate and adaptive immune responses (1). Early after infection with *L. monocytogenes*, macrophages secrete TNF and IL-12 that synergize in the activation of NK cells to secrete IFN-γ. IFN-γ in conjunction with TNF leads to robust macrophage activation, resulting in a lysteriocidal state of macrophages (2). The absolute necessity of TNF and IFN-γ for both activation of macrophages and survival of mice infected with *L. monocytogenes* has been previously shown (3–5). Although TNF and IFN-γ can induce direct antibacterial activities of macrophages by controlling antimicrobial effector pathways (6), the molecular mechanisms of the lysteriocidal activity of TNF and IFN-γ are not fully understood. Following uptake into host cells, the fate of *L. monocytogenes* depends upon the events taking place within the phagolysosomal compartment. In properly preactivated cells, *L. monocytogenes* are rapidly killed. However, in quiescent host cells *L. monocytogenes* lyse the phagosomal membrane by virtue of its pore-forming toxin, listeriolysin, and two phospholipases, PIIaA and PIIbC, and escapes into the cytoplasm, thereby avoiding the phagolysosomal degradation machinery. The signaling cascades and the actual effector mechanisms responsible for the killing of *L. monocytogenes* are not fully understood. Because of its localization within the phagolysosomal compartments and its TNF-IFN-γ-dependent activation (7–10), we hypothesized that acid sphingomyelinase (ASMase)$^3$ might be part of the bacteriocidal effector mechanisms against *L. monocytogenes*.

In this study, we show that ASMase-deficient (ASMase$^{-/-}$) mice are highly susceptible to infection with *L. monocytogenes* and *Salmonella typhimurium*. Evidence is provided that despite intact oxidative effector mechanisms, ASMase$^{-/-}$ macrophages are impaired in the killing of *L. monocytogenes*, which allows unrestricted spread of infection.

Materials and Methods

**Mice**

Breeding pairs of mice (outbred mice at a 129Sv × C57BL/6 background) heterozygous for ASMase deficiency (11) were kindly provided by R. Kolesnick (Memorial Sloan-Kettering Cancer Center, New York, NY; originally obtained from E. H. Schuchmann, Mount Sinai School of Medicine, New York, NY). Mice backcrossed six times to the C57BL/6 background were obtained from D. R. Green and T. Lin (La Jolla Institute for Allergy and Immunology, San Diego, CA). Mice were kept under specific pathogen-free conditions and were heterozygously backcrossed to the C57BL/6 strain to the eighth generation at the animal facilities of the Medical Center, University of Cologne (Cologne, Germany). Infection experiments were performed with ASMase$^{-/-}$ mice and wild-type littermates 6–10 wk old.

**Bacteria and infection of mice**

*L. monocytogenes*, strain EGD, serotype 1/2a, was kindly provided by C. Kocks (Institute for Genetics, Köln, Germany). Following in vivo passage, single colonies of the bacteria were expanded in brain-heart infusion (BHI) medium and aliquots of log-phase growing cultures were kept frozen at −80°C. For each experiment, an aliquot was freshly thawed, grown over-night in BHI medium, resuspended in fresh BHI medium, and harvested during mid-log phase. Bacteria were washed in PBS once and the density of the bacteria was estimated by OD measurement at 600 nm before adjusting the inocula to the desired density. *L. monocytogenes* were injected i.p. in 0.5 ml of PBS; serial dilutions of the inocula were plated on blood-agar plates to quantify the CFU.

*S. typhimurium*, strain ATCC 14028, were grown in Luria-Bertani medium with 0.3 M sodium chloride. After diluting an overnight culture 1/100, bacteria were harvested in the mid-log phase and inoculated i.v. at doses of 10$^5$ CFU/0.3 ml per mouse.

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$^3$ Abbreviations used in this paper: ASMase, acid sphingomyelinase; BHI, brain-heart infusion; PEC, peritoneal exudate cell; HKLM, heat-killed *L. monocytogenes*; MOI, multiplicity of infection; BSS, balanced salt solution; ROI, reactive oxygen intermediate.

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Following infection, mice were monitored daily for clinical symptoms and the time of death. At the indicated days, mice were euthanized by cervical dislocation and specimens of liver and spleen were examined for bacterial titers. Organs were homogenized in water with 0.1% Triton X-100 and 10-fold serial dilutions were plated on blood-agar plates. The plates were incubated at 37°C and 24 h later the CFU were counted to determine the CFU/g organ.

**Histopathology and immunohistochemistry**

Liver and splenic tissue samples of the same organs that were tested for bacterial titers were fixed with 4% formaldehyde containing 1% acetic acid and embedded in Paraplast X-TRA (Sherwood Medical, St. Louis, MO). Deparaffinated sections were stained with H&E, periodic acid-Schiff reaction, and Trichrome stain (all obtained from Sigma-Aldrich, Taufkirchen, Germany) according to standard protocols. For immunohistochemistry, a panel of polyclonal Abs was used: rabbit anti-*L. monocytogenes* serum (kindly provided by C. Kocks, Institute for Genetics), rabbit anti-human myeloperoxidase (DAKO Diagnostika, Hamburg, Germany), rabbit anti-human CD3, cross-reactive with murine CD3 (DAKO Diagnostika), rabbit anti-lysozyme (Biogenesis, Poole, U.K.), and rabbit anti-inducible NO synthase (Affinity BioReagents, Golden, CO). For Ag retrieval, deparaffinated sections were treated with a commercial Target Unmasking Fluid (DAKO Diagnostika) at 98°C for 20 min in a microwave oven. Incubation with primary Abs was performed overnight at 4°C. Specifically bound primary Abs were detected using a highly sensitive peroxidase- or phosphatase and...
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FIGURE 4. Immunostaining (red) of L. monocytogenes Ag in hepatic lesions of an ASMase−/− mouse (A) and a wild-type littermate (B) 4 days after infection. There is a remarkable difference of bacterium-specific Ab decoration between the KO mutant (A) and the control animal (B). Indirect immunostaining with FITC-conjugated anti-rabbit Ig detection system (Envision; DAKO Diagnostika, and Histofine, Nichirei, Tokyo, Japan, respectively). Peroxidase activity was revealed with the diaminobenzidine or the aminoethylcarbazole technique (diaminobenzidine plus and aminoethylcarbazole plus substrate kits; DAKO Diagnostika) and phosphatase activity using naphthol-AS-Bi-phosphate and New Fuchsin (Fuchsin plus substrate-chromogen; DAKO Diagnostika) as substrate. Endogenous avidin-binding activity was reduced by pretreatment of the sections with avidin and biotin solutions (Zymed Laboratories, San Francisco, CA). Finally, sections were counterstained with hemalum and permanently coverslipped.

Differential leukocyte counts were performed on Pappenheim-stained blood films according to standard laboratory procedures.

Measurement of proinflammatory cytokines

At the indicated days after infection, mice were bled and serum was prepared to determine the level of IFN-γ by ELISA (R&D Systems, Wiesbaden, Germany). Additionally, peritoneal exudate cells (PEC) were prepared from mice by peritoneal lavage with PBS 2 days after infection. PEC were incubated in microtiter plates at a density of 2 × 10^6/ml with medium alone or with 2 × 10^7/ml heat-killed L. monocytogenes (HKLM). Following 24 h of incubation, supernatants were harvested and stored at −20°C until the measurement of IFN-γ by ELISA (R&D Systems).

Killing of L. monocytogenes by macrophages in vitro

In 2-ml screw cap tubes, 2.5 × 10^6 resident or proteose-peptone-activated PEC were infected at a multiplicity of infection (MOI) of 1.0 with L. monocytogenes in 1 ml of balanced salt solution (BSS) supplemented with 5% normal mouse serum. After 15 min of rotating the mixture of cells and bacteria, extracellular bacteria were washed away by four wash cycles with BSS buffer and an additional passage of the sample through a 30% sucrose layer. After completing the wash procedure, an aliquot was drawn from the infected cell culture and plated on blood agar dishes to determine the initial bacterial burden (time 0). After various periods of time, aliquots were analyzed for bacterial growth.

Phagocytic activity of granulocytes and macrophages

The phagocytic activity of PBL was tested with Phagotest according to the instructions of the supplier (Orpegen, Heidelberg, Germany). Phagocytosis of L. monocytogenes by resident peritoneal macrophages (4 × 10^6) were co-incubated in 2-ml cryotubes with viable, FITC-labeled L. monocytogenes (12) at a MOI of 10 in the presence of 5% normal mouse serum with end-over-end rotation. At indicated times, aliquots were harvested and washed three times in ice-cold PBS to remove extracellular bacteria. The percentage of FITC-positive peritoneal cells was determined by flow cytometry.

FIGURE 5. Microscopic analysis of liver lesions in ASMase−/− mice (C and D) and wild-type littermates (B and D) 2 (A and B) and 4 (C and D) days after infection with L. monocytogenes, respectively. Two days after infection, the ASMase-deficient animal (A) demonstrates a large necroinflammatory lesion with predominantly granulocytic infiltration, an adjacent intravascular thrombus (T), and an extended area of acute liver necrosis (arrows). Note the microvesicular vacuolation of the hepatocytes due to cytoplasmic sphingomyelin storage. The wild-type littermate (B) exhibits a smaller inflammatory lesion (arrow) associated with much less hepatocellular necroses 2 days after infection. Four days after infection in ASMase−/− mice (C), multiple and irregularly shaped necroinflammatory liver lesions infiltrated by neutrophils and few macrophages are surrounded by intensively eosinophilic bands of hepatocytes undergoing necrosis. In contrast, the liver lesion (arrow) of a wild-type animal 4 days after infection (D) is characterized by the predominance of histiocytes and the absence of ongoing hepatocellular necroses. H&E; magnification, ×170.
Production of reactive nitrogen and oxygen intermediates

The production of nitrite by peritoneal macrophages activated with IFN-γ and triggered with TNF or LPS was determined by using the Greiss reagent (12). Superoxide production of macrophages activated with PMA was measured by the reduction of ferricytochrome c (12). Macrophages were collected from the peritoneal cavities of untreated mice or from animals treated by i.p. injection of protease-peptone or HKLM. The oxidative burst accompanying phagocytosis of immune complexes by PMN was determined with the Fc OxyBURST reagent (Molecular Probes, Eugene, OR) according to the instructions of the supplier. PMN were identified by flow cytometry using Grl-specific mAb (BD PharMingen, San Diego, CA).

Statistical analysis

For statistical analysis, Student’s t test was applied to the data.

Results

Susceptibility of ASMase−/− mice to L. monocytogenes

ASMase−/− mice and their wild-type littermates were infected by i.p. injection of viable L. monocytogenes covering a range from 1 × 10^3 to 1 × 10^7 CFU/mouse (Table I). Infectious doses greater than 1 × 10^6 CFU proved to be lethal for all mice. Following infection with 1 × 10^6 CFU of L. monocytogenes, all ASMase−/− mice succumbed between days 3 and 5 after infection while wild-type littermates died from day 5 on and 50% survived, indicating an LD₅₀ of ~10⁶ CFU (Fig. 1A). An infectious dose of 1 × 10⁵ CFU (Fig. 1B) killed 60% of ASMase−/− animals, revealing an LD₅₀ for these mice below 10² CFU. Only 1 of 15 wild-type littermates died following infection with an infectious dose of 10³ CFU and all wild-type mice survived infection with 10⁵ CFU (Table I and Fig. 1). Heterozygous mice were as resistant as wild-type mice at all infectious doses (data not shown). Together these results show a dramatically enhanced susceptibility (~100-fold) of ASMase−/− mice to L. monocytogenes.

The striking susceptibility of ASMase−/− mice to L. monocytogenes raised the question whether this holds true for other intra- or extracellular bacteria. As shown in Fig. 2, the susceptibility of ASMase−/− mice was also enhanced to infection with 10⁵ CFU of S. typhimurium, another facultative intracellular bacterium. It should be noted that the enhanced susceptibility of ASMase−/− mice to S. typhimurium was less dramatic when compared with L. monocytogenes. When mice were infected with a prototypical extracellular bacterium, Staphylococcus aureus, no differences between ASMase−/− and wild-type mice were observed (data not shown). These results suggest that ASMase is involved in defense mechanisms mainly against intracellular rather than extracellular bacterial pathogens.

As expected, the bacterial burden in both liver and spleen of L. monocytogenes-infected ASMase−/− mice was consistently greater than in organs of wild-type littermates. At day 2 after infection, the number of CFU of L. monocytogenes in the liver of ASMase−/− mice was 24-fold greater compared with that of wild-type mice (Fig. 3). This difference increased to 10⁴-fold at day 3 (data not shown). This overwhelming bacterial load in ASMase−/− mice could be readily visualized by staining liver sections with a L. monocytogenes-specific rabbit antiserum, e.g., on day 4 after infection. Within the liver of ASMase−/− mice, the many disseminated and extended inflammatory foci contained masses of Ab-decorated bacteria, whereas in the liver of wild-type mice only small numbers of Listeria were detectable in the fewer and much smaller lesions (Fig. 4).

Two days after infection in the livers of ASMase−/− mice, already numerous and large necroinflammatory foci were present consisting of central accumulations of granulocytes surrounded by a broad margin of hepatocellular necrosis. Other extended areas of liver necrosis were not associated with inflammatory changes but were obviously dependent on severe thromboses occurring in the larger tributaries of the central vein (Fig. 5A). In sharp contrast, wild-type livers showed only few and circumscribed necroinflammatory foci and only minor intravascular thrombotic lesions which did not lead to circulatory disturbances (Fig. 5B). In the spleens of the same mice, the typical L. monocytogenes-induced necrotizing splenic lesions that extended from the periarteriolar lymphatic sheaths of the white into the red pulp and were characterized by

![Figure 6](http://www.jimmunol.org/) Secretion of IL-1β and IL-6 by L. monocytogenes-elicited PEC restimulated with HKLM in vitro. ASMase−/− (■) or wild-type mice (□) were infected i.p. with 5 × 10⁴ CFU of L. monocytogenes. Two days after infection, PEC were collected, pooled, and restimulated in vitro with HKLM at a bacteria:cell ratio of 10:1. Following 24 h of incubation, the contents of IL-1β and IL-6 were determined by ELISA. Data shown are the means and SE of three independent experiments (p values: IL-1β, p > 0.05; IL-6, p > 0.05).
depletion of lymphocytes, deposition of fibrinoid, and prominent granulocytic infiltration appeared much more extended in ASMase<sup>−/−</sup> than in wild-type mice (data not shown). Furthermore, the mutant animals exhibited many more and considerably larger thrombi of the red pulp sinuses.

On day 4 after infection, necroinflammatory foci in the liver of ASMase<sup>−/−</sup> mice were apparently in a state of ongoing expansion, forming extended and coalescing areas of liver necrosis, while the fewer, circumscribed, and more granulomatous inflammatory lesions in the liver of wild-type animals appeared to have already reached the stage of resolution and parenchymal reorganization (Fig. 5, C and D). At this time point, the spleens of ASMase<sup>−/−</sup> mice presented with an almost complete effacement of their tissue architecture whereas in wild-type mice the inflammatory foci were already resolving (data not shown).

**Analysis of the host response to infection with L. monocytogenes**

The early death of ASMase<sup>−/−</sup> mice after *L. monocytogenes* challenge suggested a defect of the innate immune response which controls *L. monocytogenes* in wild-type mice early during the course of infection. The 100-fold increased susceptibility of ASMase<sup>−/−</sup> mice to infection with *L. monocytogenes*, the early death of these mice, and the massive necrotic decay of the liver could be secondary to the inability of granulocytes, macrophages, or NK cells to secrete IFN-γ.

**FIGURE 7.** ASMase<sup>−/−</sup> mice show enhanced serum levels of IFN-γ corresponding to high bacterial burden in liver and spleen. ASMase<sup>−/−</sup> (■) and wild-type mice (□) were infected i.p. with 2.5 × 10<sup>3</sup> CFU of *L. monocytogenes*. Two days after infection, serum samples were collected to measure the concentrations of IFN-γ (A). After bleeding the mice, livers (B) and spleens (C) were removed to determine the bacterial burden as CFU/g. Individual liver and spleen titers are depicted to allow assignment of the corresponding IFN-γ readings to the individual mouse. Means and SE for each group. Additionally, the *p* values for the comparison ASMase<sup>−/−</sup> vs wild-type group are included in each panel. The correlation coefficients for the linear regression of IFN-γ serum levels vs bacterial burden in the spleen were *r*<sup>2</sup> = 0.878 for ASMase<sup>−/−</sup> mice and *r*<sup>2</sup> = 0.853 for wild-type mice. The correlation coefficients for the linear regression of IFN-γ serum levels vs bacterial burden in the liver were *r*<sup>2</sup> = 0.983 for ASMase<sup>−/−</sup> mice and *r*<sup>2</sup> = 0.733 for wild-type mice.
cytokines needed for the activation of the innate immune system to curb the early spread of *Listeria* (2, 13).

The secretion of IL-1β and IL-6 by peritoneal exudate macrophages prepared 2 days after infection of mice with $5 \times 10^6$ CFU of *L. monocytogenes* was determined. As shown in Fig. 6, ASMase$^{-/-}$ and wild-type macrophages produced comparable amounts of IL-1β and IL-6 in response to HKLM, suggesting that cytokine secretion was intact in ASMase$^{-/-}$ macrophages. Along these lines, comparable levels of IFN-γ were detected in the sera of both ASMase$^{-/-}$ and wild-type mice (Fig. 7A). Indeed, IFN-γ serum levels seemed proportionally raised depending on the bacterial burden in the organs (compare individual mice in Fig. 7A with B and C and note the results of the regression analysis given in the legend of Fig. 7).

The fast, uncontrolled spreading of *L. monocytogenes* in ASMase$^{-/-}$ mice is not due to a numerical alteration of granulocytes and macrophages in these animals since differential blood counts of peripheral venous blood revealed no differences between ASMase$^{-/-}$ and wild-type mice (data not shown). Disturbances of the recruitment of granulocytes to infectious foci were ruled out by two lines of evidence. First, histologic analysis of the liver revealed massive infiltration of infectious foci by granulocytes (Fig. 5). Second, analysis of the percentage and absolute numbers of Gr1$^+$ cells among the PEC 2 days after i.p. infection with $5 \times 10^6$ CFU confirmed that the recruitment of innate immune cells into sites of infection was at least as efficient in ASMase$^{-/-}$ mice as in wild-type animals (Fig. 8). Moreover, we could not detect any differences in macrophage recruitment into the peritoneal cavity in this experiment (data not shown). Although the recruitment of granulocytes and macrophages to the site of infection appeared to be intact in ASMase$^{-/-}$ mice, the possibility remained that these cells were functionally defective. The elimination of bacteria by professional phagocytes requires uptake, phagolysosomal fusion, and killing of the bacteria, which involves reactive oxygen intermediates, reactive nitrogen intermediates, and proteases. To identify a possible functional defect of ASMase$^{-/-}$ phagocytes, we first tested the ability of bacterial uptake by ASMase$^{-/-}$ macrophages. The phagocytic activity of ASMase$^{-/-}$ resident peritoneal macrophages was identical to that of wild-type cells when tested with FITC-labeled, viable *L. monocytogenes* (Fig. 9).

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The ability of ASMase$^{-/-}$ and wild-type macrophages to kill *L. monocytogenes* or to prevent its multiplication was examined using resident and protease-peptone-induced peritoneal macrophages. Resident macrophages were not capable of restricting the growth of *L. monocytogenes* regardless of their ASMase status (Fig. 10). However, protease-peptone-activated wild-type macrophages were able to restrict the multiplication of *L. monocytogenes* for up to 4.5 h after infection. In contrast, protease-peptone-stimulated macrophages of ASMase$^{-/-}$ mice were neither able to reduce the amount of viable bacteria early after infection nor could they prevent the proliferation of the bacteria at later time points.
ASMase−/− macrophages ingest L. monocytogenes as well as wild-type macrophages.

In wild-type mice the interplay of granulocytes, macrophages, NK cells, and T lymphocytes orchestrated via various cytokines is necessary for overcoming acute primary infections with Listeria. For a successful defense against L. monocytogenes, secretion of IFN-γ by NK cells in response to macrophage-derived TNF is indispensable (13). Two days after infection, we detected in the sera of both ASMase−/− and wild-type mice comparable amounts of IFN-γ. A comparison of the bacterial burden in the liver and the serum levels of IFN-γ of individual mice revealed that ASMase−/− mice respond to the enhanced bacterial burden harbored in their organs with an enhanced level of IFN-γ. These data
indicate that neither the sensing of the bacterial burden nor the activation and cytokine secretion of macrophages nor the secretion of IFN-γ by NK cells are disturbed in ASMase−/− mice. Furthermore, ASMase−/− macrophages prepared from mice infected with L. monocytogenes secreted IL-1β and IL-6 in response to HKLM in vitro in amounts comparable to wild-type macrophages. These data are consistent with the observations of Manthey and Schuchman (15), who found that the signaling in bone marrow-derived murine macrophages in response to TNF, IL-1β, and IFN-γ was not altered in the absence of ASMase. Taken together, our data exclude an involvement of ASMase in the secretion of cytokines by two rather different cell types of the innate immune system in vivo and ex vivo, regardless of whether these cytokines are secreted via microvesicles (16) or endolysosomes (17) due to their lack in a leader sequence, i.e., IL-1β, or whether they are secreted by virtue of a leader sequence as is the case of IL-6 (18).

The enhanced susceptibility of ASMase−/− mice to L. monocytogenes infection is explained best by the inability of ASMase−/− macrophages to kill phagocytosed L. monocytogenes. Despite treatment with protease-peptone in vivo, macrophages from ASMase−/− mice did not acquire listeriocidal potency, whereas wild-type macrophages reduced the infectious inoculum and were able to curb the multiplication of the bacteria for at least 4.5 h after infection in vitro. Despite the inability to kill L. monocytogenes, ASMase-deficient macrophages or granulocytes produced both reactive nitrogen and oxygen intermediates. Although NO and ROI might not suffice, Shiloh et al. (19) have reported that these molecules are required for intracellular control of Listeria. Macrophages from mice double deficient in NADPH oxidase and NO synthase 2, the enzymes responsible for the production of superoxide and NO, respectively, are unable to kill virulent Listeria. The in vivo challenge of these KO mice, however, showed a bimodal response in that one-half of the mice restricted the number of bacteria as well as wild-type mice (19), indicating that additional anti-Listeria effector mechanisms exist that act in parallel to ROI and NO.

Our observations that ASMase−/− macrophages fail to kill L. monocytogenes yet produce adequate amounts of ROI and NO confirm and extend a report from Endres et al. (20), who measured normal production of ROI and NO in highly Listeria-susceptible TNF-R55-deficient mice. In addition Vazquez-Torres et al. (21) recently described that macrophages of TNF-R55-deficient mice produce abundant quantities of reactive oxygen and nitrogen species in response to Salmonella but nevertheless exhibit poor bactericidal activity. In their model, TNF-R55-deficient macrophages failed to fuse NADPH oxidase-containing vesicles with Salmonella-containing vacuoles. Because of its ability to alter the lipid composition of vacuolar membranes, ASMase has been implicated in membrane processes like vesicle fission and fusion (22). Thus, it might be worth while to investigate the role of ASMase in cytokine-induced vesicle traffic and fusion events.

Reeves et al. (23) have recently propelled the idea that proteases function as nonoxidative antimicrobial effector mechanisms. They found that the massive influx of ROI into bacteria- or Candida-containing endocytic vacuoles is accompanied by an influx of K+ ions. Within the vacuole the resulting rise in ionic strength leads to the release of granule proteins from the sulfated proteoglycan matrix. Among these granule proteins, which are activated by the release from the matrix, are elastase and cathespin G that were identified to be responsible for the killing of Candida albicans and S. aureus, respectively. As a working hypothesis, ASMase might be involved in a similar sequence of events, when cytokine-activated ASMase intraphagosomally produces ceramide that in turn activates cathepsin D, or other proteases, which might contribute to the killing of L. monocytogenes.

A detailed examination of the subcellular and molecular events leading to the enhanced susceptibility of ASMase−/− mice to L. monocytogenes and other facultative intracellular bacteria will shed new light into early cell autonomous defense mechanisms against microbial pathogens.

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